K21 Determination of Clonidine in Postmortem Specimens by LC/MS/MS

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The attendee of this presentation will be introduced to the procedural details of a LC/MS/MS methodology for measurement of clonidine in postmortem specimens. In addition, postmortem clonidine concentration data from a series of cases will be presented and the significance of these findings will be discussed.

The impact of this presentation includes an improved analytical approach for the detection of clonidine in postmortem specimens and documentation of additional postmortem clonidine concentration data to the forensic toxicology community to better facilitate interpretation.

Clonidine Hydrochloride (Catapres, Clorpres, Combipres, Duraclon), an imidazoline derivative synthesized in the early 1960s, is primarily prescribed as an antihypertensive agent available in both oral form and as a transdermal patch. Clonidine acts as an alpha2-adrenergic receptor agonist resulting in reduced sympathetic outflow from the central nervous system, thus leading to a reduction in blood pressure. Recently clonidine has been indicated as continuous epidural infusion for treatment of severe pain in cancer patients that is not relieved by opiate analgesics alone. Other uses for clonidine include treatment of anxiety and attention-deficit hyperactivity disorder (ADHD), as a preoperative sedative, and treatment of withdrawal from narcotics and nicotine. Oral dosages of clonidine hydrochloride are available in 0.1 mg, 0.2 mg, and 0.3 mg tablets with typical daily doses from 0.2 to 0.6 mg/day. Therapeutic plasma concentrations vary between 0.7 and 3.8 ng/mL.

The primary adverse effects of clonidine are dry mouth and sedation. Due to its hypnotic effect, clonidine has been used to incapacitate and subsequently rob victims, and suggested as an effective agent in drug facilitated sexual assault. Blood clonidine concentrations in the reported cases of chemical submission ranged from 13 – 68 ng/mL. Clonidine may also impair driving especially when taken with alcohol and other sedative drugs. Symptoms of overdose include early hypertension followed by hypotension, bradycardia, respiratory depression, and hypothermia. Children are reported to be especially susceptible to the sedative effects of clonidine. A serum concentration of 3.5 ng/mL produced unconsciousness in one child. After intensive treatment, a 28-year-old male survived a 100 mg overdose of clonidine and exhibited a peak plasma concentration of 370 ng/mL.

Clonidine is not detected in routine toxicological screening methods used in most laboratories conducting postmortem analysis. The low therapeutic and toxic concentrations encountered in blood coupled to the need for derivatization prior to GC/MS analysis contribute to this fact. The aim of this work is to develop a simple and sensitive LC/MS/MS analytical procedure for detection of underivatized clonidine and document the occurrence of clonidine in postmortem specimens from casework conducted in our laboratory. Cases for this study were selected based upon clonidine appearing in the list of medications collected at the scene.

Clonidine and 7-aminoflunitrazepam, as the internal standard, were isolated from alkaline postmortem fluid and tissue homogenate by extraction with n-butyl chloride. The n-butyl chloride fraction was evaporated to dryness. The residue was reconstituted in mobile phase, washed with hexane (saturated with mobile phase) and submitted for analysis by LC/MS/MS. Liquid chromatography was performed using an Agilent 1100 with Agilent Zorbax Eclipse XDB-C8 column. The column was 150mm by 4.6mm with 5 micron film. The mobile phase was an isocratic mixture of methanol (55%) and water (25 mmol ammonium formate pH 3.0) (45%). The LC flow (0.25 ml/min) was directed into an electrospray ionization source and mass spectral analysis was performed with an API 2000 triple quadrupole mass spectrometer (Applied Biosystems).

Qualitative identification of clonidine was established by monitoring two transition ion pairs, m/z 229.9/212.8, m/z 229.9/171.8. Quantitative analysis monitored the peak areas of the m/z 229.9/212.8 transition for clonidine and the 284.1/135.0 transition for 7-aminoflunitrazepam. A five point curve from extracted blood calibrators, ranging from 0.4 to 4.0 ng/mL, was generated, r2 =0.9988. The established limit of detection was 0.1 ng/mL. Of nine cases screened thus far, postmortem blood clonidine concentrations ranged from 0.64 – 6.9 ng/mL.

Clonidine, LC/MS, Postmortem

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